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Original article

Microstructure of the water spider (*Argyroneta aquatica*) using the scanning electron microscope

Jung-Hoon Kang [a], Yu-Jin Lee [a], Bu-Kyun Oh [a], Sung-Kyung Lee [a], Bo-Ra Hyun [a], Bong-Woo Lee [b], Yong-Gun Choi [c], Kye-Soo Nam [d], Jong-Deock Lim [a,*]

[a] *National Research Institute of Cultural Heritage, Cultural Heritage Administration, Daejeon, Republic of Korea*
[b] *Division of Research and Planning, Korea National Arboretum, Pocheon, Gyeonggi-do, Republic of Korea*
[c] *Korean Institute of Biospeleology, Daejeon, Republic of Korea*
[d] *Daejeon Science High School, Daejeon, Republic of Korea*

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ABSTRACT

This study is aimed to identify the external features of the water spiders (*Argyroneta aquatica*) collected from "The Natural Monument No. 412 Yeoncheon Eundaeri Water Spider Habitat" through observation of their microstructures using a scanning electron microscope. There is no study on the microstructures of the water spiders excluding several studies on protection plans and ecological investigations, thus giving this study considerable academic significance. Based on the scanning electron microscopy analysis, the water spider has eight simple eyes, and both of its lateral simple eyes are stuck together. A lateral bump was confirmed on the upper jaw, and the pedipalps had six joints and the legs had seven joints. The abdomen and sternum of *A. aquatica* have more hairs compared with those of land spiders, and its structure shows an elongated area of contact with the air bell so that the air bell can become attached to the abdomen better.

Introduction

The Natural Monument Number 412 Yeoncheon Eundaeri Water Spider Habitat, which covers a total area of 50,508 m^2, is a flat land comprising mainly rice paddies at 693-18 Eundae-ri, Jeongok-eup, Yeoncheon-gun, Gyeonggi-do, South Korea, and it is an isolated ecosystem—there are no corridors leading to different environments such as mountains (Cultural Heritage Administration, 2007). Moreover, the habitat is composed of three main zones.

Although topographically it is a flat land, two of the zones in the northeast have slightly lower areas than the adjacent rice paddies so the water flows toward the water spider habitat (National Research Institute of Cultural Heritage, 2009). The environment of the habitat is a shallow swamp with almost no stream velocity, and its water is acidic with a pH of 5.90–6.20. There are aquatic plants, protozoa, water flea, damselfly larvae, mosquito larvae, and other food sources (Kim and Lim, 2011).

There is only one genus and one species of water spiders (*Argyroneta aquatic*; Clerck 1757) in the world, and they are found in Korea, Japan, China, temperate zones of Europe, Siberia, and Central Asia. Its life span is 1 year—similar to that of all other spiders in Korea (National Research Institute of Cultural Heritage, 2009). The water spider produces air bells and spends almost all of its entire life on such activities as feeding, mating, spawning, development, and growth under water, making it a distinctive spider with a unique life cycle (Locket et al., 1974; Aakra and Dolmen, 2003; Nielsen and Hauge, 2007; Lee, 2008). In Korea, water spiders are observed only in the wetlands in Eundae-ri, Jeongeok-eup, Yoncheon-gun, Gyeonggi-do, and this entire area was designated as the natural monument (Namgung et al., 1996; Kim, 2002).

Unlike most land spiders, the male water spiders are larger than their female counterparts. This sexual size dimorphism is an advantage for water spiders, in that larger males have mobility advantages over smaller ones when moving under water, giving the males a decided edge when finding their mates (Schütz and Taborsky, 2005).

The previous studies on water spiders were only on methods on how to protect them and ecological examinations, and not morphological research through observations of the microstructure of these spiders. Therefore, a scanning electron microscope (SEM) was used to observe the microstructure of water spiders in order to

* Corresponding author. Tel.: +82426107630.
E-mail address: dinostudy@outlook.com (J.-D. Lim).
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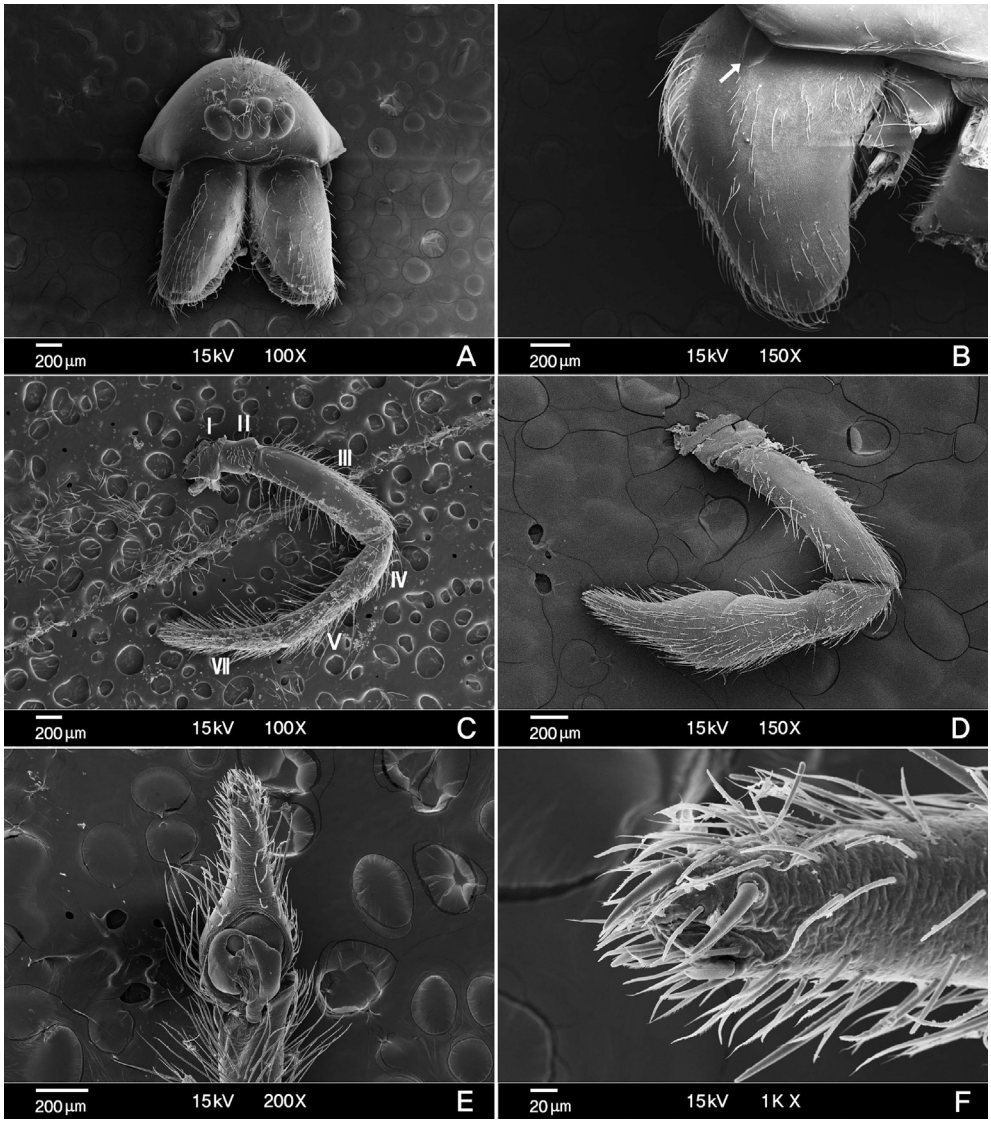

Figure 1. *Argyroneta aquatica*. (A) Head (front). (B) Upper jaw (side). Arrow: lateral condyle. (C) Pedipalp (female). (D) Pedipalp (male). (E) Reproductive organ on inside of pedipalp (male). (F) Pedipalp end joint.

identify their external characteristics. This study was carried out to identify the structure(s) that could have an effect on the behavior and ecology of the water spiders. The findings can be used as fundamental data for continued studies on the ecology and reproduction of water spiders in the future.

Materials and methods

Sample preparation

A total of 16 water spiders (8 males and 8 females) were used for the SEM analysis. All water spiders used for the study were collected between May 2014 and July 2014 at the Yeoncheon-gun Eundae-ri Water Spider Habitat for Water Spider Reproduction and Conservation Research.

Pretreatment

Fixation

The part of the sample to be observed was cut and placed in 2.5% glutaraldehyde and left overnight, then washed with 1× phosphate buffer for 10 minutes. This was repeated three times and after removing the supernatant liquid, 1% OsO_4 was added, and it was allowed to stand for 40–60 minutes. Then, 1× phosphate buffer was used again to wash the sample for 10 minutes; this was repeated three times prior to fixation.

Dehydration

The sample was placed in 50% ethanol and left to stand for 20 minutes; next, it was removed and placed in 60% ethanol, left to stand for 20 minutes, and then removed again. This process was carried out at 70%, 80%, and 90% sequentially, and finally, it was placed in 100% ethanol and allowed to stand for 20 minutes, a process that was repeated three times.

Substitution

The dehydrated samples were placed in hexamethyldisilazane for 30 minutes, a process that was repeated twice; then, the samples were dried for about 1 day.

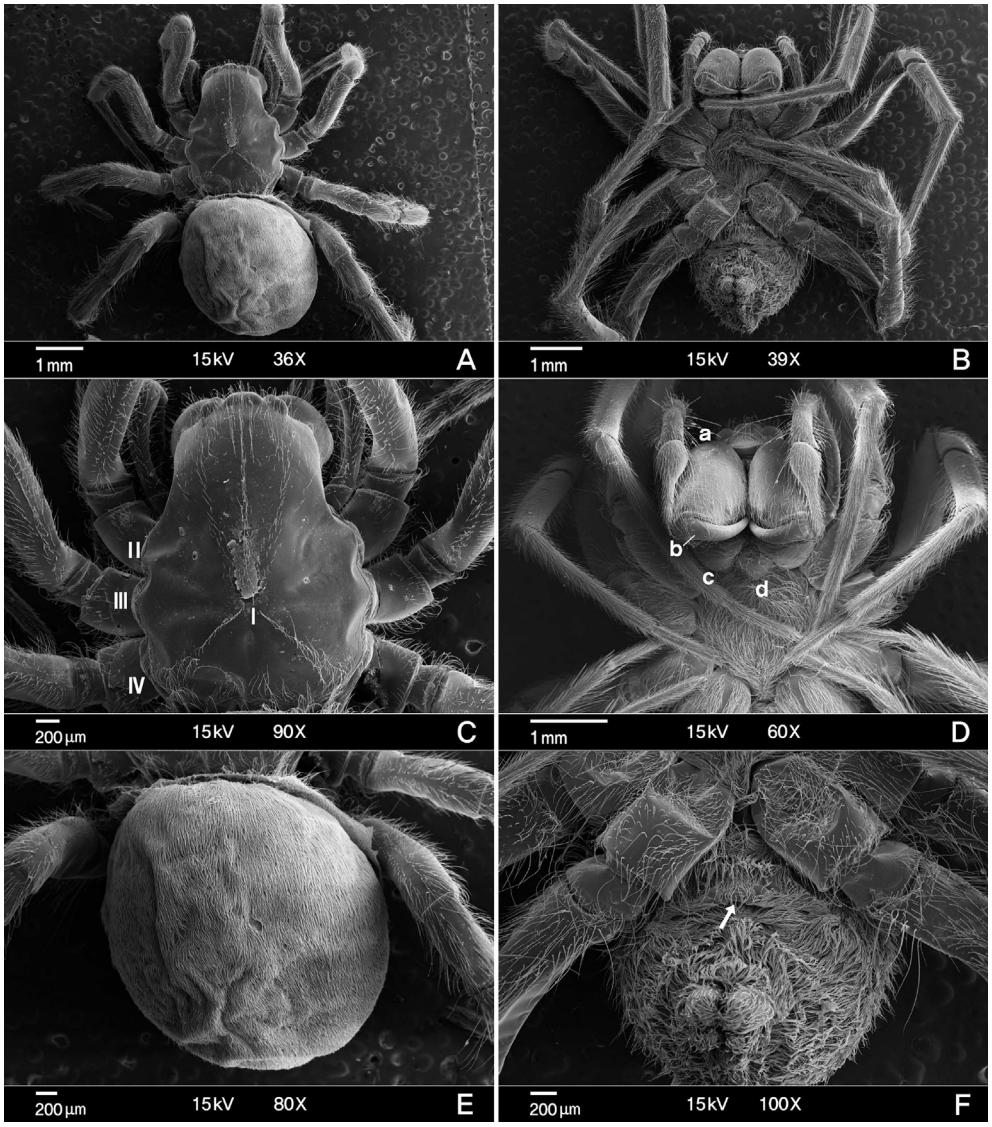

Figure 2. *Argyroneta aquatica.* (A) Entire body (upper). (B) Entire body (bottom). (C) Cephalothorax (upper). (D) Cephalothorax (bottom): (a) upper jaw; (b) fangs; (c) upper lip; and (d) lower lip. (E) Abdomen (upper). (F) Abdomen (bottom). (F) External reproductive organ (arrow) (♀).

Slide preparation

The pretreated and dried water spiders were placed on a glass slide with carbon tapes to examine the morphological characteristics of the water spider.

Scanning electron microscopy

Slides with the pretreated dried samples were coated with gold (Sputter Coater: BAL-TEC SCD 005, Balzers, Liechtenstein). An SEM (LEO 1455VP, Leo Electron Microscopy Ltd., Cambridge, UK) was used for filming and analysis from August 2014 to September 2014.

Result and discussion

The external features of every major part of the water spiders (as observed through SEM) are discussed in the following subsections.

Size

The water spiders had an average size of 6.75 mm for the matured samples, and the average size of males was 6.97 mm and that of the females was 6.53 mm.

Color

The color of the cephalothorax and the legs is similar, and the carapace is brown or reddish-brown without any pattern.

Head

The water spider has eight simple eyes. The median four simple eyes are square shaped. The lateral simple eyes from both sides are stuck together so that they look like one big oval eye (Figure 1A), and the left and right eyes are slanted approximately 20–30° to the left and right, respectively.

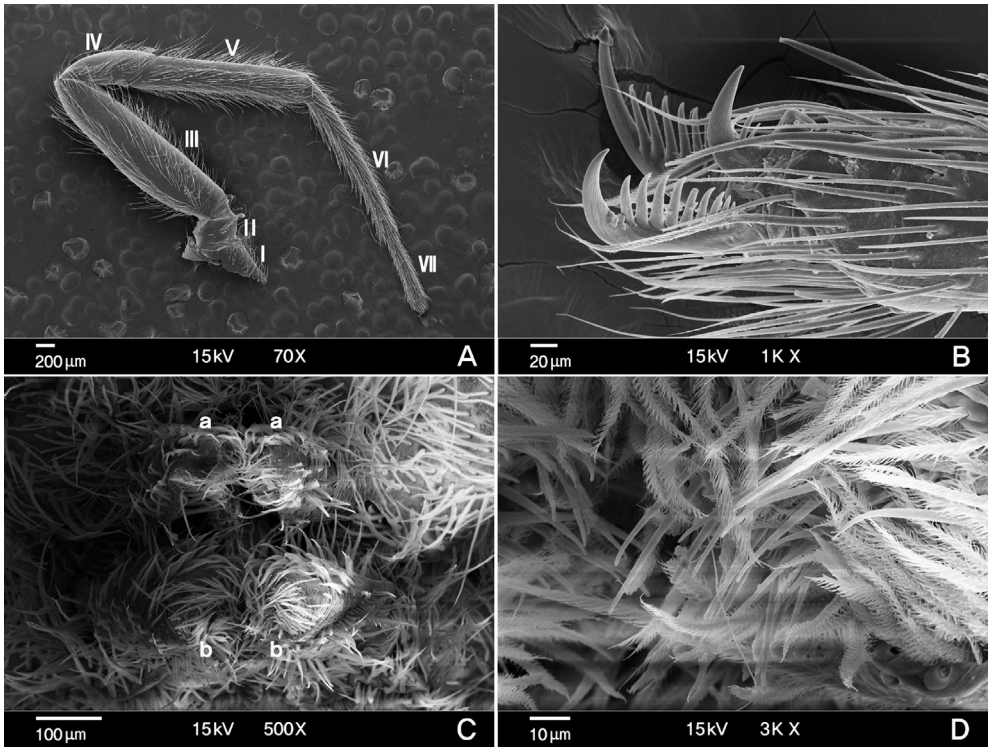

Figure 3. *Argyroneta aquatica*. (A) Leg (leg 3). (B) Claw (leg 4). (C) Spinneret: (a) front spinneret and (b) rear spinneret. (D) Close-up view of spinneret.

Mouth

The constituents for taking food—the upper jaw (Figure 2Da), fangs (Figure 2Db), upper lips (Figure 2Dc), and lower lips (Figure 2Dd)—were confirmed. When capturing food, water spiders secrete neurotoxins and protein dissolution toxins using their fangs, with the poison glands connected to their upper jaw. A bump was found on the upper jaw (Figure 1B, arrow). Not much is known about the function of the lateral condyle, but different species of arachnids also have them, so it is used for classification.

Pedipalp

The pedipalp is made up of six joints—coxa (I), trochanter (II), femur (III), patella (IV), tibia (V), and metatarsus (VII) (Figure 1C). When compared with the leg, it does not have a tarsus (VI). It is located on each side of the lips, and it is morphologically similar to that of the female (Figure 1C); moreover, there are many bristles and serrated hairs on the metatarsus (Figure 1F). The males have a metatarsus that swells on the bottom in the shape of a club (Figure 1D), and as the metatarsus develops, it matures and is used for mating. At the end of the metatarsus of the male's pedipalp, a reproductive organ is formed for mating (Figure 1E), and when this reproductive organ is inserted into the external reproductive organ (Figure 2F, arrow) in the lower part of the mature female's abdomen, mating is completed. In the case of the reproductive organs (pedipalp) of males and the external reproductive organ of females, they are species-specific behavior. In addition to its role in reproduction, the pedipalp plays an auxiliary role when spiders catch their prey.

Thorax

In the thorax of the cephalothorax, aside from the median furrow (I) in the thorax as the center, there is a furrow (II) that goes out in a frontward direction and a furrow (III) in a horizontal direction, as well as a furrow (IV) that stretches backward, making a total of four furrows (Figure 2C).

Legs

One leg is made up of six joints—coxa (I), trochanter (II), femur (III), patella (IV), tibia (V), tarsus (VI), and metatarsus (VII) (Figure 3A). When compared with the pedipalp, the metatarsus has more and longer serrated hairs, and there are three claws in the shape of a comb (Figure 3B). Using these claws and the numerous serrated hairs, the water spider can easily go up vertical surfaces or slippery structures.

Abdomen

The abdomen is oval shaped and has a gray-brown or dark brown color, and there are many hairs on the abdomen and the sternum (Figure 2D–F). The hairs found in these areas are much more numerous compared with those of land-dwelling spiders, and it is judged that water spiders use them for attaching air bells to their abdomen and moving within the water. The detailed structure of the hairs shows that they are long and softly serrated (Figure 3D), and the numerous serrated hairs expand the contact surface with air bells, thereby allowing the spider to attach air bells to its abdomen. At the end of the abdomen, there are a total of four spinnerets—the front spinnerets (Figure 3Ca) and the rear spinnerets (Figure 3Cb)—for making spider webs in water. The liquid that makes spider webs produced by the spinning gland in the abdomen is changed into the form of a spider web through the spinneret. The abdomen of the water spider is covered with hair, and no tracheal spiracle was observed.

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